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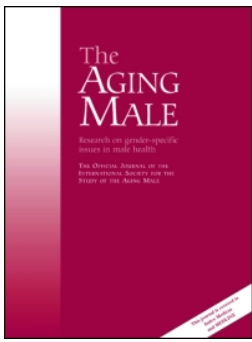
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To cite this article: Ricardo Aparecido Baptista Nucci , Ana Caroline de Souza Teodoro , Walter Krause Neto , Wellington de Assis Silva , Romeu Rodrigues de Souza , Carlos Alberto Anaruma & Eliane Florencio Gama (2017): Effects of resistance training on liver structure and function of aged rats, The Aging Male, DOI: [10.1080/13685538.2017.1350157](https://doi.org/10.1080/13685538.2017.1350157)

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Published online: 11 Jul 2017.



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## Effects of resistance training on liver structure and function of aged rats

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### ABSTRACT

The aging process may cause negative physiological changes. However, exercises as resistance training (RT) have been considered an important intervention to attenuate these changes. Additionally, liver plays an important role in blood glucose homeostasis in exercise.

**Aim:** This study aimed to analyze the effects of RT on the liver components of aged animals.

**Methods:** Male Wistar rats were divided into two groups: 24 months' group (CONTROL); and group submitted to a progressive RT protocol for 16 weeks (EXERCISE). Both groups were sacrificed at 24 months.

**Results:** We observed a decrease in blood flow due to the practice of resistance exercises. Besides, our results showed that hepatic tissue plays an important role in glycemic homeostasis during RT. In addition, RT increased mitogen capacity of hepatocytes.

**Conclusions:** Our study showed many implications for the knowledge about the effects of strength training on old animals' liver.

### ARTICLE HISTORY

Received 21 June 2017  
Revised 29 June 2017  
Accepted 29 June 2017  
Published online 10 July 2017

### KEYWORDS

Liver; aging; resistance training; hepatic structure; exercise

### Introduction

With increased longevity, the quality of life and general health of the older segments of society have become important topics of discussion and research [1].

The aging process may cause muscular weakness, increase the risk of falls and cognitive impairment in the elderly population [2–5]. However, older people who maintain a regular exercise routine have attenuated the physiological changes of the aging process [2–5].

Resistance training (RT) has been highlighted as an important factor for increasing strength and muscle mass in both young and older subjects [2,6,7]. Thus, the goal of achieving a RT program in the elderly is to maintain, and if possible, increase muscle and bone mass, thereby contributing to the maintenance of daily living activities [2,8,9]. Besides, in exercise, the hepatic tissue plays an important role in blood glucose homeostasis by glycogenolysis and gluconeogenesis [10].

Although many physiological effects of regular physical activity have been described in the literature [2–9], there is still unclear evidences about the effects of RT on hepatic structure and function in the elderly.

### Methods

All experimental procedures conformed to the guiding principles of the American Physiology Society and were approved by the Ethical Committee of São Judas Tadeu University under the protocol number 001/2013.

To carry out this study, 12 male Wistar rats were used. The rats were randomly divided into two groups: (A) sedentary rats with 24 months of age (CONTROL,  $n = 6$ ); (B) rats with 20 months that were subjected to RT for 16 weeks, totaling 24 months of age (EXERCISE,  $n = 6$ ). The body weight (BW) was measured at the beginning of each week.

All animals in the RT group underwent a pre-adaptation to the training protocol and equipment for three days. The equipment used to carry out the strength training program with the animals was a vertical ladder made of wood with iron steps. The height of the equipment (ladder) is 110 cm (43.3 inches) with an inclination angle of 80°. The top of the equipment had a plastic box for the accommodation of the animals in the interval between sets [11,12].

The training protocol was progressive with the load being adjusted every week. The load was composed of lead weights that were attached to their tails with a velcro tape. The animals were supposed to climb the ladder to reach the resting area at the top that was considered one repetition. The adaptation process was three days with four repetitions every day.

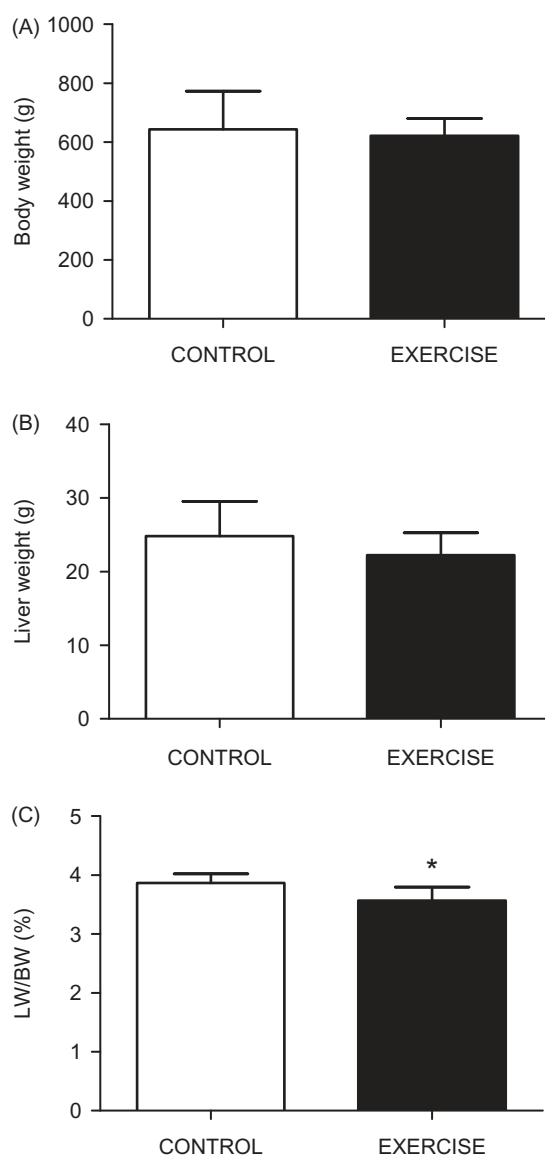
The rats in exercise group were trained once a day throughout three days per week for 16 weeks with a rest interval of 60s between repetitions. Each training session consisted of six to eight climbs. Over the course of 16 weeks, the amount of weight carried by each rat was equivalent to 50% of its body weight (BW). The BW was measured at the beginning of each week of the experiment and the new weight to be carried by the animals during that week was adjusted according to their BW [11,12]. No external stimulus was necessary so that the animals conduct the training.

After the experiments, the animals were sacrificed by CO<sub>2</sub> method and the liver was removed. Then fragments of liver were fixed in 10% buffered formalin and dehydrated in an increasing series of alcohols. They were cleared in xylene, embedded in paraffin, sectioned in 5  $\mu$ m thick sections and stained with hematoxylin and eosin (HE) and periodic acid of Schiff (PAS) for light microscopy analysis.

The histological sections stained with HE were used to analyze the area and the numerical density of hepatocytes and other liver cells using the Axio Vision software (version 4.9.1). For numerical density, we counted all the cells (hepatocytes or other liver cells, e.g. Kupffer and Stellate cells without discrimination) in each field [13].

In HE histological sections we analyzed, through stereological methods, the volume density of lobular parenchyma components (capillaries sinusoids, peri sinusoidal spaces and biliary ducts) and non-lobular parenchyma components (portal spaces, veins and center-lobular branches of blood vessels). Finally, the sections stained with PAS were used to analyze the percentage of glycogen present in the tissue [13]. For the determination of numerical density of glycogen, Image J software (National Institute of Health, version 1.45s) was used. It was done by counting the number of structures present in the photographic field of microscopic field. The structure which touched the lines to the right and upwards on the screen were disregarded, while those which that touched the lines on the left and downward were considered [14].

Statistical analyzes were performed using GraphPad Prism 5.0 software (GraphPad Prism, Inc., San Diego,



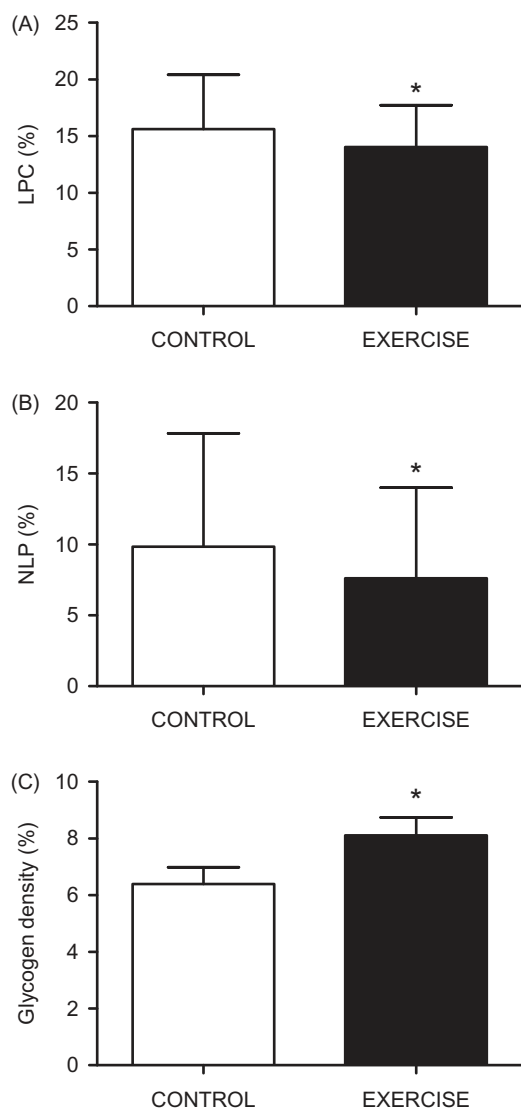
**Figure 1.** (A) body weight (BW g). (B) Liver weight (LW g). (C) Relative liver weight times 100 (LW/BW %). Data shown as mean  $\pm$  SD. \* $p < 0.05$ .

CA, USA). Student's *t*-test was used to compare groups and summary data reported as mean values  $\pm$  standard deviation. A  $p < 0.05$  was considered statistically significant.

## Results

No significant difference was observed between groups for body weight and liver weight (Figure 1). However, relative liver weight times 100 (LW/BW %) decreased with exercise ( $p < 0.05$ ).

Figure 2 shows a sharp decrease in both lobular parenchyma components (LPC %) and non-lobular parenchyma components (NLP %) in the group submitted to RT ( $p < 0.05$ ). Furthermore, we highlight in our



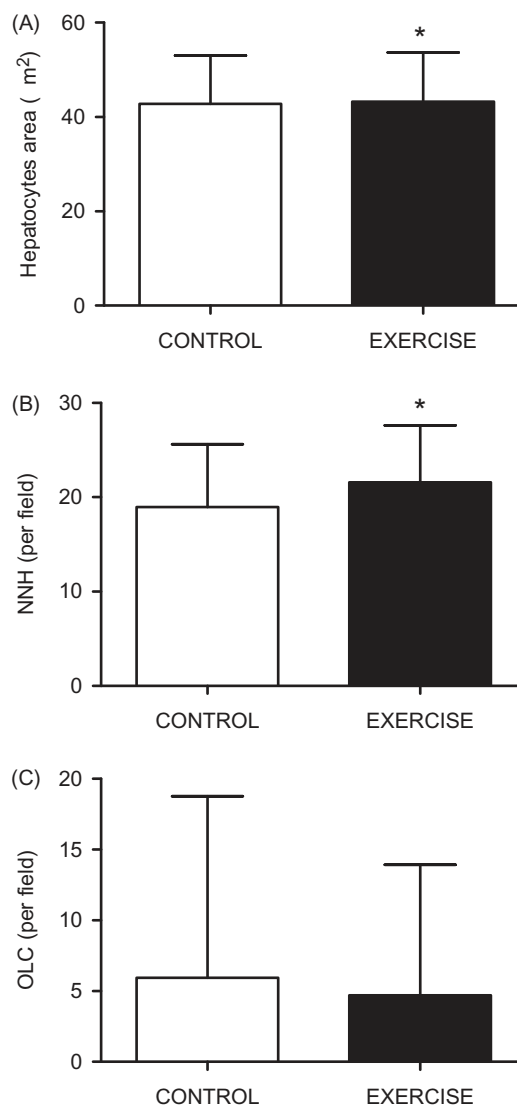
**Figure 2.** (A) lobular parenchyma components (LPC %). (B) Non-lobular parenchyma components (NLP %). (C) Glycogen density (%). Data shown as mean  $\pm$  SD. \* $p < 0.05$ .

study a sharp increase in hepatic glycogen in the exercise group ( $p < 0.05$ ).

Figure 3 shows a subtle, but statistically significant, increase in hepatocytes area ( $\mu\text{m}^2$ ) with strength training, in which exercise has increased hepatocytes area in 1.18% ( $p < 0.05$ ). In addition, we observed an increase in number of nuclei of hepatocytes (NNH) per field with RT ( $p < 0.05$ ). The results indicate an 13.87% increase of NNH per field with RT. However, other liver cells (OLC) per field, had no significant difference between groups.

## Discussion

Our study analyzed the effects of RT on the liver components of old animals. Observing Figure 1, we can see that strength training did not changed



**Figure 3.** (A) Area of hepatocytes ( $\mu\text{m}^2$ ). (B) Numerical density of hepatocytes (NNH) per field. (C) Other liver cells (OLC) per field. Data shown as mean  $\pm$  SD. \* $p < 0.05$ .

body weight. In our experimental model, we did not use a high-intensity RT, which could explain the body weight maintained in the exercise group [15].

We can see that the absolute weight of the liver did not change with RT. In order to have no doubts about these results, we performed the relative liver weight, established by the ratio of the total liver weight (absolute weight) divided by the final BW of the animal times 100. Regarding to the relative liver weight (Figure 1(C)), we observed that RT is a factor that decreases liver mass. Additionally, in Figure 2, we suggest, using LPC % and NLP %, that RT may reduce blood flow in the liver. According to van Wijck et al. [16] these results may be related to splanchnic hypoperfusion. Drainage of the splanchnic area is covered by the superior mesenteric vein, inferior mesenteric vein, and the portal vein, which return the venous

blood to the heart via the liver and subsequently the inferior vena cava [16,17]. The splanchnic vasculature promotes vasodilatation or constriction via regulation of the mesenteric vascular resistance by neuroendocrine, humoral, and paracrine mechanisms [16,18]. During exercise, the release of norepinephrine near the  $\alpha$ -adrenoreceptors of the sympathetic nervous system induces splanchnic vasoconstriction, thereby increasing total splanchnic vascular resistance [16,17,19,20]. The blood is rapidly redistributed from the splanchnic area to be used for the perfusion of tissues with increased activity during exercise, such as heart, lungs, active muscle, and skin [16,21,22].

Moreover, exercise is a condition that results in fast mobilization and redistribution of substrates for the performance of muscle activity, which numerous changes in hormone secretion and metabolism become necessary for the maintenance of homeostasis [23]. In the exercise, the hepatic tissue plays an important role in maintaining blood glucose [10]. Our result, furthermore, provides evidence that hepatic glycogen stores undergoes adaptations to resistance exercise, as the trained group had more glycogen reserves than the sedentary group.

After a finite number of divisions, primary cell cultures enter a state of replicative senescence in which oxidants and antioxidants plays an important role in cellular signaling to further mitogenic stimulation [24]. If oxidative stress and the ability to respond appropriately to it is important in ageing, then it follows that factors that increase resistance to stress should have anti-ageing benefits and lead to enhanced life span [24,25].

Exercise increases oxygen consumption and reactive oxygen species (ROS) generation and, therefore, can enhance oxidative damage to nucleic acids in cells [25–28]. On the other hand, it has been well recognized that regular physical activity has health benefits such as reducing risk and progression of cardiovascular diseases, type 2 diabetes mellitus, cancer and neurodegenerative diseases [25,29–31]. Paradoxically, these diseases are suggested to be induced and exacerbated by ROS [25]. Additionally, Oliveira et al. [32] showed that animals submitted to aerobic exercise had the lower percentage of polyploids nuclei. Polyploidy results from incomplete mitotic cycles, which is tightly related to the aging process [32–34]. Our results in Figure 3 showed that the number of hepatocytes nuclei per field (NNH per field) were higher in animals submitted to RT, suggesting that exercise increase hepatocytes mitotic cycles increasing its numerical density and maintaining its area across age.

These results may be related to the protective effect of regular exercise even on hepatic cells.

In conclusion, there are three major findings in the present study. First, a decrease in blood flow due to the practice of resistance exercises suggesting a splanchnic hypoperfusion. Second, our results provide evidence that hepatic glycogen stores undergo adaptations to resistance exercise, demonstrating that liver plays an important role in glycemic homeostasis during RT. Finally, a numerical increase in hepatocytes with RT due to a potential antioxidant effect of regular exercise on liver cells.


## Acknowledgements


The authors wish to thank Dr. Carlos Alberto Anaruma, José Roberto Rodrigues da Silva and Eduardo Custódio, “Julio de Mesquita Filho” São Paulo State University, São Paulo, Brazil, and Magna Soares, Butantan Institute, São Paulo, Brazil, for technical support.

## Disclosure statement

The authors report no declaration of interest.

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